5 Peptide Emergence, Evolution and Selection on the Primitive Earth

II. The Primary Pump Scenario

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Abstract We propose a dynamic scenario for the emergence and evolution of peptides on the primitive Earth, through a molecular engine (the primary pump), which works at ambient temperature and continuously generates, elongates and complexifies sequential peptides. This new scenario is based on a cyclic chemical reaction sequence that could have taken place on tidal beaches; it requires a buffered ocean, emerged land and a nitrosating atmosphere. We show that the primitive Earth during the Hadean may have satisfied all of these requirements.

This scenario is not necessarily what actually happened, but it represents a global approach of peptide prebiotic synthesis, and most of its parts are accessible to experiment. As it develops, it may open up a gateway to the emergence of homochirality and the catalytic activities of peptides.

5.1 From N-carbamoyl Amino Acids (CAA) to Peptides

5.1.1 Introduction

The hypothesis of the prebiotic RNA world (Gilbert 1986; Gesteland et al. 1999) is hampered by the infeasibility of producing these macromolecules without the aid of enzymatic catalysts or modern organic synthetic tools (Joyce and Orgel 1999). In the absence of any satisfactory scenario to explain the origin of peptides, the coemergence of RNA and peptides as suggested by Kauffman (1993) or De Duve (1998), remains itself a fragile hypothesis. The prebiotic emergence of peptides meets two major obstacles: the activation of amino acids, then the possibility of forming long peptide chains under aqueous conditions, namely the only reasonable prebiotic solvent until now. Three different approaches have been developed in this direction.

The first approach focuses on peptide synthesis under extraterrestrial conditions (e.g. interstellar ice grains), through the analysis of meteoritic content as the only available record. This approach remains disappointing: only the Gly-Gly dipeptide has actually been detected in the Murchison and Yamoto-791198 meteorites (Shimoyama and Ogasawara 2002). Experimental models of interstellar ice do not show evidence of peptide formation.1

In our opinion, the formation of peptides in an extraterrestrial ice analogue claimed by Munoz Caro et al. (2002) results from a hasty interpretation of predictable results.
The second consists in studying the behaviour of amino acids at high temperature. At 100–180°C racemic compounds are obtained with insufficient evidence for the presence of peptide bonds (Rohlfing 1976; Fox and Dose 1977). Wet/dry cycles at 25–100°C, in the presence or absence of clays or silicates, lead to dipeptides and traces of racemic trimers to pentamers (Lahav et al. 1978; Rode et al. 1997). At 100°C in the presence of NiS/FeS and thiols to simulate volcanic zones or undersea vents, only dipeptides and traces of racemic tripeptides are detected (Huber and Wächtershäuser 1998). This approach is therefore highly contested (Bengston and Edstrom 1999).

The third approach investigates activated α-amino acids (esters, thioesters, N-carboxy anhydrides) or activating agents (carbonyl diimidazoles or carbodiimides) with amino acids. It is more encouraging since it effectively allows for oligocondensation under smooth, nonracemising conditions, whether in homogeneous aqueous solution, on the surface of rocks, (Paecht-Horowitz and Eirich 1988; Orgel 1989, 1998; Bohler et al. 1996; Weber 1998), or in double-layer membranes (Luisi et al. 2000); however, it has not yet led to the expected sequential peptides2 (Luisi 2000). For this approach to be credible we need to understand how activated molecules, which are highly reactive and sensitive to hydrolysis, could have been continuously formed on the primitive Earth. The relevance to prebiotic chemistry of activating agents such as carbonyl diimidazole or dicyclohexyl carbodiimide, or of amino acid N-carboxy anhydrides (NCA) remains contested (Liu and Orgel 1998; Blair and Bonner 1980, 1981).

Therefore the question of the prebiotic formation of long peptides remains open. We shall address this question by investigating a cyclic reaction scheme occurring in an open system out of equilibrium. The dynamics of such system (which is an important feature of living organisms) is very likely to result in self-organisation, as highlighted by, e.g. Nicolis and Prigogine (1977). It has been the object of numerous, mostly theoretical investigations in many fields – including the origins of life. Meanwhile, very few experimental studies have been published on the behaviour of such open chemical systems, despite their fundamental interest. Investigations (including experimental) on the prebiotic emergence and (self-) organisation of complex chemical reaction schemes, including their dynamical aspects, seems therefore important.

In the previous chapter we showed that the conditions that existed on the primitive Earth could have selectively led to N-carbamoyl amino acids (CAA), which have, however, been considered for long as an evolutionary cul-de-sac under prebiotic conditions. In this chapter we shall examine a possible activation pathway from CAA to sequential peptides through amino-acid N-carboxy anhydri-

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2 In such experiments amino acids identified after acidic hydrolysis (6N HCl, 120°C for 6h) probably do not actually originate from the hydrolysis of peptides, but rather of α-amino nitriles, namely the Strecker precursors of observed α-amino acids.

2 In this chapter, sequential peptides are referred to as peptides made of different amino-acid residues, (of optionally known sequence), oppositely to homopeptides in which all residues are identical.
drides (NCA), based on a dynamic process, that might also have contributed to the emergence of homochirality and of catalytic activity. Our attention will be focused on the relevance of the required conditions with respect to the primitive Earth environment.

5.1.2 The Primary Pump

Our approach of the prebiotic emergence of peptides is based on a cycle of chemical reactions forming the basis of a molecular engine, called by us the primary pump and shown in Fig. 5.1 (Commeyras et al. 2001, 2002).

**First cycle.**

The first step (1) is the formation of N-carbamoyl amino acids (CAA) either by reaction of free amino acids with cyanate, or in other ways (see previous chapter). The second step (2) is the concentration of these CAA by water evaporation. This could have taken place on the shores of Hadean continents, at

![Fig. 5.1. The primary pump: a molecular engine leading to the production of evolutionary sequential peptides, starting from CAA and from NCO\(^{-}\), NO, O\(_2\), HCO\(_3\)Na, H\(_2\)O]
low tide. In the dry phase, NO$_x$ from the atmosphere (see Sect. 5.2.3 and previous chapter) react with the CAA (nitrosation), leading to NCA through the cyclisation of unstable nitroso urea intermediates, step (3). This reaction is quantitative at ambient temperature, producing stoichiometric amounts of nitrogen, water and nitrous/nitric acid together with NCA. In such acidic medium NCA degradation is relatively slow; within a few hours they undergo hydrolysis ($t_{1/2} \approx 1$ h) into $\alpha$-amino acids, thus being recycled. At step (4) the rising tide quickly puts NCA into contact with ocean water (this is also conceivable for tidal pools or lagoons), the pH of which (5–7) allows peptide formation from NCA in competition with their hydrolysis, step (5). This step is complete within a few minutes. Afterwards, the so-produced peptides can react with cyanate to give N-carbamoyl peptides, step (6); this reaction occurs as easily as amino-acid N-carbamoylation. Over longer time in the hydrosphere, peptides and N-carbamoyl peptides undergo slow hydrolysis, step (7); this leads to shorter peptides, eventually down to $\alpha$-amino acids. Meanwhile both undergo N-carbamoylation.

• Next cycles.

The reaction of NCA with N-carbamoyl peptides is impossible, thus theoretically preventing peptide elongation in subsequent drying/wetting cycles. However, when N-carbamoyl peptides arrive on the shore and are dried, they are immediately unprotected by reaction with NO$_x$ (Collet et al. 1999; Lagrille et al. 2002), step (3). This reaction occurs under the same conditions as NCA formation from CAA. Therefore at the next high tide, unprotected peptides are elongated by reaction with newly formed NCA.

Such successive elongation/partial hydrolysis cycles may have allowed peptide chemical evolution. The intrinsic rate of peptide formation is higher than that of peptide hydrolysis, which should be favourable to peptide accumulation; however, actual rates are highly dependent on the reactant fluxes and, moreover, on the dry/wet alternation period.

When considering peptides, it is difficult not to associate homochirality with emergence, as hypothesized by George Wald (1957). It is well known that the extraterrestrial input of $\alpha$-amino acids was not totally racemic, see for instance Pizzarello and Cronin (2000). If these nonracemic $\alpha$-amino acids played a role in the synthesis of peptides, then their (low) enantiomeric excesses may have been amplified. Such amplification phenomena have been demonstrated by Blair and Bonner (1980), however, in organic solvents. If in addition, amino acids or CAA racemise faster than peptide-chain residues, then peptide prebiotic synthesis

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$^3$ In this chapter NO$_x$ is referred to as the result of mixing NO and O$_2$ in any ratio, which can equivalently be obtained in other ways, e.g. mixing NO and NO$_2$.

$^4$ In aqueous solution it is likely that free amino acids epimerise slower than peptide residues. However, the latter may epimerise slower than some amino-acid derivatives such as CAA (recent, unpublished results of our group are consistent with this possibility), or even slower than free amino acids/peptide end-residues if epimerisation catalysts are involved.
may have progressed towards homochirality. A great deal of work still needs to be done to test this hypothesis.

5.2 Environmental Requirements

Each step of the so-called primary pump has been studied carefully and can be considered as well known. Nevertheless, for the primary pump to produce peptides, all steps must be well-controlled. Under prebiotic conditions, is it conceivable that, spontaneously, the correct sequence of steps took place and gave peptides? What are the minimal requirements to fulfill such a goal? Without any doubt, oceans and emerged land are required and they must be in contact with an atmosphere containing nitrogen oxides. The time window for this scenario lies between $-4.4\text{ Gyr}$ and $-3.8\text{ Gyr}$. The following discussion will consider the probability of these environmental requirements, with special emphasis on the pH of the aqueous phases, which is critical in our scenario.

5.2.1 Primitive Earth

The oldest terrestrial rocks recognized so far are the Acasta gneisses in Canada, dated at $-4.030 \pm 0.003\text{ Gyr}$ (Bowring and Williams 1999), which consist of a tonalite, trondhjemite and granodiorite suite (TTG) that is typical of the primitive Archaean continental crust. The oldest known terrestrial materials are zircon crystals, extracted very recently from Jack Hill metaquartzites (Australia) and dated at $-4.404 \pm 0.008\text{ Gyr}$ (Wilde et al. 2001). Their chemical characteristics demonstrate that they crystallized in TTG-like magmas, thus establishing the existence of a stable continental crust as early as 4.4 Gyr ago. In addition, hafnium and neodymium isotopic studies in both old zircons and early Archaean TTG (McCulloch and Bennet 1993; Wilde et al. 2001) show that, when these materials were generated, significant volumes of continental crust had already been extracted from the terrestrial mantle. Based on these isotopic data, McCulloch and Bennet (1993) calculated that between 8 and 15% of the volume of the present-day continental crust had already been extracted prior to $-4.0\text{ Gyr}$.

Petrogenetic studies performed on the Archaean TTG continental crust have shown that it was generated by partial melting of hydrous basalts (Martin 1986, 1987). Available water lowers the temperature of basalt solidus by several hundred degrees, and is therefore absolutely necessary for melting basalt and generating TTG. Basalts, generated at great depth by anhydrous melting of mantle peridotite, contain only very small amounts of water. Today they incorporate water through alteration by seawater, for instance in midocean-ridge systems. As an essential consequence, the existence of a Hadean continental crust implies

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\[ \varepsilon_{\text{Hf}} \] (for Hf) and \( \varepsilon_{\text{Nd}} \) (for Nd) were both found to be positive: \( \varepsilon_{\text{Hf}} \) (resp., \( \varepsilon_{\text{Nd}} \)) represents the difference between the isotopic ratio \( ^{176}\text{Hf}/^{177}\text{Hf} \) (resp., \( ^{143}\text{Nd}/^{144}\text{Nd} \)) of a rock and that of chondrites, taken as the reference.
that huge volumes of liquid water must have been available on the surface of the primitive Earth. Mojzsis et al. (2001) and Wilde et al. (2001) have analyzed the oxygen isotopic composition of the 4.4- and 4.3-Gyr zircons: the $\delta^{18}O$ (5.4–15.0‰) clearly indicates that the source of the magma in which these zircons crystallized had already strongly reacted with a hydrosphere. This demonstrates that oceans existed as early as 4.4Gyr ago on the Earth’s surface; this conclusion is also supported by the fact that the Isua gneisses in Greenland (dated at 3.865Gyr) consist of sediments accumulated in an aquatic environment.

The existence of Hadean beaches acted upon by ocean tides (the presence of the Moon at that time is now widely accepted) is therefore shown to be a consistent hypothesis.

5.2.2 Primitive Atmosphere

The abundance of atmospheric nitrogen oxides ($NO_x$) is a limiting factor for the efficiency of nitrosation and thus for the production of peptides through the primary pump. Unfortunately, the nature (pressure, temperature, composition) of the prebiotic atmosphere remains obscure in the absence of geological archives, preventing us from addressing quantitatively this point. Still, we can discuss the availability of $NO_x$ considering the few constraints on the primitive terrestrial environment.

The formation of $NO_x$ is considered to occur through two successive steps: (1) formation of nitric oxide NO through activation of $N_2$; (2) formation of higher $NO_x$ species by reaction of NO with atomic or molecular oxygen. The major oxygen source is photodissociation of CO$_2$, with a mixing ratio highly dependent on CO$_2$ pressure (Rosenqvist and Chassefière 1995; Selsis et al. 2002). In a 1-bar CO$_2$ atmosphere, O$_2$ pressures up to 20mbar can be maintained at a steady state in this way, though this would depend upon there being no O$_2$ consumption at the Earth’s surface. Such an uptake would almost certainly have occurred, through the oxidation of rocks and reducing volcanic gases (Selsis et al. 2002), the amount of which is unknown, however. While it has been suggested that the early upper mantle was more reducing than it is today (Pavlov et al. 2000), its present oxidation state could have been reached as early as 4.3Gyr ago (Tolstikhin and Marty 1998). We performed computer simulation of atmosphere models under photochemical activation, using the computer code PHOEBE (photochemistry for exobiology and exoplanets, Selsis et al. 2002), starting from $N_2 + CO_2$ background atmospheres including water vapour at thermodynamic equilibrium. For initial mixtures of 0.8bar of $N_2$ plus 0.2bar (A) or 3.2bar (B) of CO$_2$, O$_2$ mixing ratios of $10^{-4}$ and $10^{-3}$ were found for configurations A and B, respectively, if no O$_2$ uptake is taken in account. Supposing this O$_2$ uptake to be at the same level as today, the calculated mixing ratio drops to $10^{-6}$ and $5 \times 10^{-5}$ for configurations A and B, respectively. An example of such simulation (configuration A) taking in account other NO sources (see infra) is shown in Fig. 5.2.

Concerning NO formation, the partial pressure of $N_2$ is thought to have reached a level close to its present value (0.8bar) earlier than 4.3Gyr (Tolstikhin
Fig. 5.2. Simulation of the primitive Earth atmosphere composition with the numerical code PHOEBE. The 1-bar background atmosphere of N$_2$ (80%) + CO$_2$ (20%) includes NO production by volcanic lightning (Navarro-Gonzalez et al. 1998) and Marty 1998). A high CO$_2$ partial pressure ($p_{\text{CO}_2}$) is usually assumed, although a recent study (Sleep and Zahnle 2001) claims that the formation of carbonates was so efficient on the Early Earth that CO$_2$-dominated atmospheres have occurred only for geologically brief periods following giant impacts. In our case, we assume $p_{\text{CO}_2}$ to be at least high enough to insure a mean surface temperature above 0°C. Depending on the period considered (from 4.3 to 3.6 Gyr ago), this implies a minimum $p_{\text{CO}_2}$ being in the range 100–400 mbar (Caldeira and Kasting 1992). If $p_{\text{CO}_2}$ was lower, two cases can be considered:

1. No other greenhouse gas warmed the surface and the Earth was globally frozen: surface liquid water was then restricted to the vicinity of active volcanic zones, or at the surface of ice near the equator and during the warmest day hours. The efficiency of the primary pump in such conditions has not been addressed yet.

2. The surface is warmed above 0°C by CH$_4$ produced in hydrothermal systems (Pavlov et al. 2000): In that situation, the presence of abundant reduced species in the atmosphere considerably lowers the photochemical lifetime of the NO$_x$ and inhibits the nitrosation.

Thus, a prebiotic atmosphere for which nitrosation cannot be a priori ruled out contains at least 100 mbar of CO$_2$ but no abiotic methane. This picture is compatible with our knowledge of the primitive Earth as recently reviewed by Kasting and Catling (2003).

In the abiotic early atmosphere, NO$_x$ have to be produced from N$_2$ (and not from the biogenic N$_2$O as it is now). The N–N bond energy is particularly high and consequently, short wavelengths are required for the photolysis of N$_2$ ($\lambda <$
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100nm). Under such conditions the photochemistry initiated by solar UV does not produce significant levels of NO\textsubscript{x} (Selsis 2000). Such a conclusion is valid for the present-day situation, but what about the Hadean era? Indeed, observations of young Sun-like stars (Guinan and Ribas 2000) leads to the conclusion that the soft X-ray and hard UV (XUV: 1–100nm) emission of the young Sun was very probably much higher than its present value. The luminosity in the XUV range \( L_{X} \) varies as \( L_{X}(t)/L_{X,\text{Sun}} = 6.16 \times t^{-1.19} \) where \( t \) is the age of the star in Gyr, and \( L_{X,\text{Sun}} \) the present solar XUV luminosity. The photochemistry initiated by this intense XUV flux on the early Earth still has to be explored but it surely led to a more efficient production of NO\textsubscript{x} at high and middle altitudes. However, even under such irradiation conditions and in the presence of an atmosphere without reduced species such as CH\textsubscript{4} and H\textsubscript{2}, the surface mixing ratio of cumulated NO\textsubscript{x} species do not reach \( 10^{-9} \).

Another, more efficient way to produce NO\textsubscript{x} consists in shocking up to more than 1500K a gas mixture containing N\textsubscript{2} and oxygen-bearing species (O\textsubscript{2}, CO\textsubscript{2}, H\textsubscript{2}O) and cooling it as rapidly as possible. In this case, the oxygen molecules, or atoms generated at high temperature, react with N\textsubscript{2} producing NO. Such chemistry occurs during lightning, meteors and impacts.

The efficiency of these processes in our present O\textsubscript{2}-rich atmosphere is higher than in an anoxic atmosphere where the reaction N + O\textsubscript{2} → NO + O does not occur. In the absence of O\textsubscript{2}, the efficiency depends on the ratio CO\textsubscript{2}/N\textsubscript{2}. When the ratio \( [\text{N}_2]/[\text{CO}_2] \) is above 5, the formation of NO (through the reaction N + CO\textsubscript{2} → NO + CO) becomes inefficient because of the fast recombination of N atoms into N\textsubscript{2} through the three-body reaction 2N + N\textsubscript{2} → 2N\textsubscript{2}. But when the CO\textsubscript{2} and N\textsubscript{2} abundances are comparable, the energy yield of the NO production is only 10 times lower than in an O\textsubscript{2}-rich atmosphere (Navarro-Gonzalez et al. 2001).

Navarro-Gonzalez et al. (1998, 2001) studied both experimentally and theoretically the production of NO by lightning in CO\textsubscript{2}-rich atmospheres. Considering the early volcanic activity and the number of lightning strikes associated to volcanic plumes, these authors estimated the continuous production of NO on Early Earth and found \( 3 \times 10^{9} - 3 \times 10^{10} \text{mol g}^{-1} \) for an atmosphere with \( [\text{CO}_2]/[\text{N}_2] > 0.3 \). As an upper limit, if all of these radicals could be used by the primary pump, this rate would allow a NCA production of \( 10^{-9} \text{mol cm}^{-2} \text{s}^{-1} \). Of course, a fraction of the produced NO is reduced by volcanic gases, or reacts to form HNO\textsubscript{3} that is rained out through acid rains. This continuous production occurring in the lower atmosphere could maintain an average abundance of about \( 10^{-6} \), 100 times higher than in the present-day atmosphere.

In the case of meteors, few quantitative studies have been done (see Jenniskens et al. 2000, for the meteor-induced chemistry in a CO\textsubscript{2}-rich atmosphere). NO is produced in the plasma of the meteor trails in which air temperature is briefly raised to about 5000K: NO and NO\textsubscript{2} lines have thus been observed in Leonid meteor spectra (see, for instance, Carberry et al. 2003). The NO\textsubscript{x} produced by these small-sized bodies is generated in the upper atmosphere, above
50 km and, even in the absence of detailed modelling, this source does not seem to contribute to the delivery of NO\textsubscript{x} to the lower atmosphere.

More studies are available for larger impacts because of the environmental consequences they may induce. Prinn and Fegley (1987) modelled the K/T impact\textsuperscript{6} of a 5 × 10\textsuperscript{14}-kg asteroid, or a 1.25 × 10\textsuperscript{16}-kg comet. They suggested that the explosion could have produced from 5 × 10\textsuperscript{14} to 1.2 × 10\textsuperscript{17} moles of NO, explaining by the same way the acidic content of the worldwide K/T geological layer. However, Zahnle et al. (1988) showed in a short note that Prinn and Fegley overestimated these numbers by considering an instantaneous cooling. Later, Turco et al. (1982) estimated that the Tunguska bolide (probably a comet) that disrupted in the atmosphere in 1908 generated about 3 × 10\textsuperscript{12} moles of NO between 10 and 50 km of altitude. In an early, CO\textsubscript{2}-dominated atmosphere, this would produce in a single event 3 × 10\textsuperscript{11} moles of NO, the same amount as the whole production of NO by volcanic lightning. Again, most of the NO is generated in the middle atmosphere and rained out in the form of nitric acid. Further modeling would be required to know how much NO reaches the surface. Indeed, this source may be very efficient and nearly continuous as Tunguska-like events may have occurred 0.1–1 times a year on the prebiotic Earth. In a review paper addressing the environmental consequence of large impacts, Toon et al. (1994) inferred a power law giving the mass production of NO as a function of the impact energy.

We summarized these results in Fig. 5.3, using Navarro-Gonzalez et al. (2001) for scaling the result to a CO\textsubscript{2}-dominated atmosphere. We applied the standard evolution of the impact rate: an exponential decrease with a timescale of 144 Myr (Chyba 1990) until ~3.5 Gyr ago. The impact flux 3.5 Gyr ago, equal to the present-day one, is taken from Morrison et al. (1994). We assume that the impactors are asteroids with a typical density of 3 g cm\textsuperscript{−3}, a mass distribution that is constant in time, and an average impact velocity of 20 km s\textsuperscript{−1}.

For impact energies above 10\textsuperscript{21} J (equivalent to an impactor diameter of 3 km), the induced NO is well mixed down to the surface. For smaller bodies, 40 m–3 km, the abundance of NO peaks up to 10\textsuperscript{−3} in the middle atmosphere and diffuses to the surface where it can reach 10\textsuperscript{−6} as an upper limit. Impactors with diameter below 40 m do not deliver efficiently NO to the surface. For the largest impacts (similar to those that formed the main Lunar Basins around 3.9 Gyr ago) the entire atmosphere is heated to T > 1500 K and may contain 0.1% NO, (10\textsuperscript{19} moles). Because such hot conditions obviously frustrate organic chemistry, we did not consider impacts with energy above 10\textsuperscript{25} J (equivalent to a 50-km impactor) as a source for “useful NO”. Converted in mol yr\textsuperscript{−1}, the production of NO by impactors in the 3–50 km range is quite low (< 1.3 × 10\textsuperscript{7} mol yr\textsuperscript{−1}, see Fig. 5.3), but every 10\textsuperscript{4}–10\textsuperscript{5} years, the abundance of NO reaches 10\textsuperscript{−3} and

\textsuperscript{6} Of a ca. 10-km diameter object, having occurred 65 Myr ago at the Cretaceous/Tertiary era (K/T) boundary, and plausibly responsible for the extinction of the dinosaurs. Such impact energy is estimated between 10\textsuperscript{22} and 10\textsuperscript{24} J (equivalent to 0.25 to 25 billion Hiroshima bombs).
Fig. 5.3. Nitric-oxide production by impacts. Black lines: impact frequency as a function of age, for three diameter ranges of impactors. Blue lines: corresponding production of nitric oxide. Dashed lines: extrapolation beyond 4.0 Gyr (age of oldest available Lunar cratering records)

decreases with a typical timescale of 1 yr (much shorter than the typical interval between two impacts of this size).

In summary, the continuous source of NO on the early Earth was due to volcanic lightning. In the most favourable conditions (a negligible abiotic production of methane and a moderate release of reduced volcanic species) it could have maintained an average abundance of NO around $10^{-6}$, this level being higher or lower, by at least one order of magnitude, depending on the proximity (in space and time) to volcanic plumes. Violent and stochastic impacts resulted in higher abundance (up to $10^{-3}$) decreasing slowly for months or years. From the present knowledge, we can thus conclude that, if the primary pump did contribute to the production of peptides on the prebiotic Earth, it may have been fed with NO by single and violent events (impacts or volcanic eruptions) rather than continuously. Complementary experimentation and modelling will be necessary anyway in the future to substantiate this very hypothetical scenario.

5.2.3 About the pH of Primitive Oceans

5.2.3.1 Requirements from the Primary Pump

The pH of the aqueous phase (the primitive ocean) is a critical parameter of our scenario, especially for steps (1) and (5). Concerning step (1), the N-carbamoylation of amino acids occurs at any pH comprised between 5 and 12, with a maximum rate at pH 7–8. CAA are stable over the whole pH 5–12 range. Concerning step (5), the polycondensation of NCA in water has been studied
Bartlett and Dittmer 1957; Bartlett and Jones 1957). Below pH 4, only NCA hydrolysis occurs. Above pH 4, there is a competition between NCA hydrolysis and NCA condensation on amino acids or peptides, with increasing condensation yields while increasing the pH. In the pH range 4–6.5 this competition creates the most favourable conditions for an efficient kinetic selectivity in peptide formation, thus allowing the chemical evolution of the growing peptide pool. This is important especially for the selection of homochiral peptides.

5.2.3.2 Environmental Data.

With respect to these requirements, it is important to have an idea of the pH of the primitive ocean. Due to the presence of large amounts of CO$_2$ in the primitive Earth atmosphere, (Kasting 1993; Zahnle 1998), the early ocean was probably initially quite acidic (its pH being possibly as low as 3.5). The pH should then have gradually increased to its current value (8.2) through progressive extraction of alkaline materials (Na$^+$, K$^+$, Ca$^{2+}$, etc.) from reducing rocks.

Therefore the primary pump process could have started as soon as the pH of the ocean reached 4, and could have worked until it reached ca. 6.5. This pH window is estimated to have taken place during the Hadean. Moreover, the pH of the ocean may have remained around 6.3 (pK$_A$ of carbonic acid) for a long time due to the buffer effect. More precise knowledge of this primitive-ocean pH chronology is necessary since it would allow the corresponding time windows to be refined.

5.2.3.3 pH Change During Water Evaporation (Step 2).

A possible drawback in the activation of CAA can be the acidification of the medium during evaporation: CAA are well known to convert into hydantoins under acidic conditions (usually pH $< 2.5$), what we did not observe in our experiments simulating prebiotic conditions. In fact the evaporation of an aqueous solution of pH 6.5 containing (bi)carbonate salts as the major components does not result into its acidification since carbonic acid will evaporate as well as CO$_2$ during the concentration process (in fact we even observed an increase in pH; ultimately an alkaline salt of the CAA is obtained). Therefore the possibility of hydantoin formation during step (2) appears very limited. Conversely, a part of the NO$_x$ from the atmosphere will be necessary to neutralise alkali before the nitrosation takes place.

5.3 Investigation of the Primary Pump

In the above section we have seen that α-amino acids N-carboxy anhydrides (NCA), may have been synthesized in a fairly continuous way on the primitive
Earth. This lends credence to the argument for peptide synthesis through activated α-amino acids (see introduction) as it argues in favour of the prebiotic relevance of NCA, which are also the major monomers for the preparation of synthetic polypeptides from laboratory to industrial scale.

At the same time our approach acquires a dynamic dimension. It gives prebiotic significance to Blair and Bonner’s results concerning the amplification of low enantiomeric excesses during polymerization of NCA, even though their results were obtained in nonaqueous solvents (Blair and Bonner 1980, 1981). It provides arguments for carrying out the necessary research to evaluate the potentialities of the primary pump. We investigated (see next Section) the different steps of the primary pump, first separately, focusing on steps (1), (3) and (5), then in a more integrated way.

5.3.1 Step-By-Step Experimental Investigation

5.3.1.1 Step (1): N-carbamoylation

Cyanate (NCO⁻) irreversibly reacts with α-amino acids to produce CAA acids. Study of this reaction shows that it can occur in aqueous media over a very wide pH range: 5 to 13 (Taillades et al. 2001), with the rate law (5.1) involving the hydrolysis of cyanate (k₀), the catalysis by carbonates (k₁), the formation of urea (k₂) and the N-carbamoylation (k₃) of amino acids (AA) (t = total: acid + base forms).

\[ v = \frac{d[NCO]}{dt} = [NCO]^{t} \times (k₀ + k₁ \times [CO₃]^{t} + k₂ \times [NH₃]^{t} + k₃ \times [AA]^{t}). \]  

(5.1)

At 50°C and pH 6.5, the N-carbamoylation rate constants k₃ were measured: 1.7 × 10⁻³ L mol⁻¹ s⁻¹ for glycine, 1.3 × 10⁻³ L mol⁻¹ s⁻¹ for valine, and 3.5 × 10⁻³ L mol⁻¹ s⁻¹ for threonine. This reaction is possible only above pH 5, with almost quantitative yields at pH 7 for cyanate to α-amino acid ratios above 1.5.

In addition to its interest for the quantification of a future computer kinetic model, this reaction might allow refinement of the moment the primary pump start-up, namely when the pH of primitive oceans reached 5 (see Sect. 5.2.3).

5.3.1.2 Step (3): CAA Nitrosation

The gaseous mixture O₂/NO reacts on CAA even in the absence of water, as shown in Fig. 5.4. (Collet et al. 1996; Taillades et al. 1999). Photo (a) shows crystals of anhydrous N-carbamoyl valine. In the presence of an excess of anhydrous gaseous mixture O₂ + NO N-carbamoyl valine is quantitatively (according to NMR analysis) converted into valine-NCA at 20°C within half an hour (photo c) (Collet et al. 1996). The middle photo (b) shows the surface of the crystal during the reaction. The bubbles that are forming are of nitrogen. The liquid consists of the water and nitrous acid produced by the reaction (step (3)). Even
under initially anhydrous conditions, the solid CAA is completely converted into solid NCA (Taillades et al. 1999).

The reaction efficiency depends on the O$_2$/NO ratio. In the absence of O$_2$ there is no reaction. When the O$_2$/NO ratio is equal to, or greater than, 1 the reactivity is below its maximum, which is observed for an O$_2$/NO ratio of 0.25 (this ratio probably remained quite low during the Hadean). The NO$_x$ gaseous mixture actually contains the species NO, O$_2$, N$_2$O$_4$ and N$_2$O$_5$ in equilibrium, the ratio of which depends on the O$_2$/NO mixing ratio. We evaluated the rate constants of CAA nitrosation in the solid-gas phase by N$_2$O$_4$, N$_2$O$_3$ (nitrosating agents present in the gaseous NO$_x$ mixture) and also by nitrous acid HNO$_2$ (a side-product of both CAA nitrosation and N$_2$O$_3$/N$_2$O$_4$ hydrolysis). The values obtained at 25°C ($k_{N_2O_4} = 1.7s^{-1}$, $k_{N_2O_3} = 10^8s^{-1}$ and $k_{HNO_2} = 0.1s^{-1}$, respectively) show the very high reactivity of N$_2$O$_3$ (Lagrille 2001): even traces of this species in the primitive atmosphere might have been sufficient for activating CAA into NCA.

Further studies will be necessary to evaluate the working limits of the primary pump, in terms of, e.g. minimum NO$_x$/CAA ratio, or NO$_x$ concentration in the atmosphere.

5.3.1.3 Step (5): Insights in Peptide Synthesis

According to George Wald’s suggestions (Wald 1957) the α-helical secondary structure of a growing homochiral polypeptide chain might ensure the selection of α-amino acids of the same chirality as those already present in the chain during its lengthening, thus preserving and increasing the homochirality of the growing chain.

These suggestions were proved by Idelson and Blout (1958) and Lundberg and Doty (1957), by studying the polymerization (in dioxane) of the NCA. Stereoselectivity was negligible at the simple peptide level and much higher for the α-helix at higher degrees of polymerization. In the same paper Wald said “If one could grow such polymers in a reversible system in which synthesis was
partly balanced by hydrolysis, the opportunity for selection would be greatly improved." Obviously, Wald was underlining the importance of water. Our results giving prebiotic status to NCA led us to carry out these studies again using water as solvent.

**Oligocondensation of NCA in water: stereoselectivity.**

NCA in aqueous solutions undergo two competitive reactions during step (5), hydrolysis and condensation (aminolysis), with different kinetic laws:

\[
v_{\text{hyd}} = k_{\text{hyd}} \times [\text{HO}^-] \times [\text{NCA}],
\]

(5.2)

and

\[
v_{\text{cond}} = k_{\text{cond}} \times [\text{R-NH}_2] \times [\text{NCA}],
\]

(5.3)

where R–NH₂ is the amine form of amino acid or peptide.

We reconsidered the work carried out by Bartlett (Bartlett and Dittmer 1957; Bartlett and Jones 1957), using capillary electrophoresis. With this technique, we directly measured the rate of NCA hydrolysis and of peptide formation (Plisson et al. 2002). Partial results for valine-NCA give

\[
k_{\text{hyd}} = 4.40 \times 10^{-4} \text{s}^{-1}
\]

for hydrolysis, and

\[
k_{\text{LL}} = 1.65 \times 10^{-2} \text{L mol}^{-1} \text{s}^{-1}
\]

and

\[
k_{\text{LD}} = 1.14 \times 10^{-2} \text{L mol}^{-1} \text{s}^{-1}
\]

for the formation of Val₂ from l-Val-NCA reacting on l-Val and d-Val, respectively.

So, in water, the hydrolysis rate of the NCA is low compared to their condensation rate. The condensation rate constant \(k_{\text{LL}}\) is much greater than \(k_{\text{LD}}\), and for the formation of dipeptide, the \(k_{\text{LL}}/k_{\text{LD}}\) ratio is already much greater than 1. This ratio must be higher for the synthesis of larger peptides (octapeptides and above).

**Fast Measurement of Peptide pKₐ.**

In water, the (pH-dependent) formation rate of peptides depends on their pKₐ, which are, however, not very well known. In our group, Cottet et al. (personal communication, to be published) have developed a fast method for measuring the pKₐ of oligopeptides using capillary electrophoresis (CE), with no need to isolate them from the mixture. Figure 5.5 illustrates the pKₐ measurement of oligoglycines.

From this information, we deduce that, in aqueous solution, longer peptides (lower pKₐ) react faster with NCA than the shorter ones (higher pKₐ), assuming their intrinsic rate constants of coupling with NCA to be identical (Fig. 5.6). This last point needs to be confirmed.

On the basis of these experimental data, we are running computer simulation to investigate possible enantiomeric amplification of the peptide pool by the primary pump.
Fig. 5.5. pK$_A$ of oligoglycines Gly$_n$ (1–9 residues) as a function of $n$ (Cottet, to be published)

Fig. 5.6. In aqueous solution, for equal concentration of peptides, the concentration of the nonprotonated amino group of the longer peptides (lower pK$_A$) is higher than the concentration of the nonprotonated amino group of shorter peptides

5.3.2 Integrated Experimental Approach: Chemoselectivity

This physicochemical approach needs to be checked by synthesis techniques because, in water, other parameters are involved in the lengthening of peptide chains, and these parameters need to be understood.

We investigated the chemoselectivity of peptide synthesis under the conditions of the primary pump, by experimentally cycling its essential steps (1–5) several times (NaHCO$_3$ buffer 100 mM, pH 6.7) containing amino acids or their $N$-carbamoyl derivatives (Commeyras et al. 2002). Results using two amino acids valine (V) and glutamic acid (E) are shown in Fig. 5.7. Many peptides are formed but not all of them. Figure 5.8 shows the distribution of peptides formed in these experiments and shows also a very effective chemoselectivity during the elongation process.

With four amino acids (glycine, alanine, valine, glutamic acid) as starting materials, MALDI-TOF analysis shows that the length of the peptides increases
with the cycle number, but insoluble peptides are formed. The insoluble fraction (separated after 7 cycles) is made up of peptides whose molecular weight is distributed around 800 daltons, whereas the mass of the soluble compounds is higher than 2000, thus evidencing the effect of additional selectivity factors, physical rather than chemical.

The stimulating aspect of this point is that a stress (here possibly due to hydrophobicity of peptide chains) separates the insoluble peptides. The only

Fig. 5.7. Peptide elongation and selection after 5 cycles under primary pump conditions, running with 2 amino acids valine (V) and glutamic acid (E). Analysis by HPLC/MS (ESI/Q-TOF)

![Peptide elongation and selection chart](image)

Fig. 5.8. Observed chemoselectivity during the formation of peptides under primary pump conditions, from 2 amino acids (V, E) as starting materials

![Chemoselectivity chart](image)
peptides capable of evolving in the short term are the soluble ones, while the others – inappropriately adapted – are “frozen” as formed, and probably slowly hydrolyzed.

In conclusion we can consider that in aqueous solution, the above-described selectivities could have considerably decreased the number of possible peptides formed \((2^n x^{n > 20} x^{x > 100})\), and have thus made this synthesis realistic. Detailed analysis of these various selectivities will require additional research.

5.4 Energy

Chemical evolution involves the interconversion of energy under its various forms, from physical: heat, electromagnetic (radioactivity, UV light, etc.), mechanic (tides, impacts, etc.) to chemical, through the formation of activated molecules, which could prefigure the increasing complexity of metabolic intermediates in the living world.

The primary-pump scenario is one example of this first step of converting energy from physical to chemical, in a permanent, continuous way: primary energy sources (heat, UV light, etc.) promote the formation of small activated molecules such as cyanic acid and NO\(_x\), then followed by anhydrides such as NCA, responsible for the polymerisation of amino acids. As an energy carrier, NCA may also have been involved in other processes. Furthermore, as chemical structures increased in complexity, other activated/activating species should have emerged. Why not therefore consider NCA as possible ancestors (another could be thioesters, see De Duve 1998) of adenosine triphosphate (ATP), the “modern”, somewhat ultimate energy carrier in today’s biological world?

The kinetic model of the primary pump shown in Fig. 5.1 could, in a future study, be broadened to include how it was fed with matter and energy as shown in Fig. 5.9.

The energy aspect, with photochemical reactions leading to the essential prebiotic molecules, for which there are already rich sources of information and methods, could be developed through the utilization of, dedicated software such as PHOEBE (photo-chemistry for exobiology and exoplanets: see Selsis et al. 2002).

Concerning the feeding of matter, the selective synthesis process of CAA is described in the previous chapter. We showed that about 50 carbonyl derivatives, combined with ammonia, methylamine, ethylamine, hydrocyanic acid, and carbonic anhydride, are sufficient to model the formation of all meteoritic \(\alpha\)-amino acids and terrestrial peptides. The mechanisms of the reactions involved in these syntheses are known, along with most of their kinetic constants (Comnemaras et al. 2003).

Although complex, only kinetic modelling of the whole system can provide understanding or insight into the evolution of such systems.
Fig. 5.9. Global view of the primary pump with matter and energy feeding.
5.5 Conclusions and Perspectives

Our primary-pump scenario appears to be the first that could have been capable of supplying sequential peptides under rather realistic prebiotic conditions. Maintained out of thermodynamic equilibrium by continuous/sufficient inputs of matter (amino acids) and energy (through activated molecules such as cyanate and NO$\textsubscript{x}$), this dissipative system has the ability to recycle its reagents and to increase the complexity of the reaction products (peptides). The minimum requirements for the prebiotic relevance of the model, namely the presence of oceans, emerged land and nitrogen oxides in the atmosphere, seem to have been fulfilled on the primitive Earth and the primary pump could work as soon as the pH of the oceans rose to 4–5. Our results underline the importance of such a buffering effect and suggest the most suitable locations for this process were beaches, rather than closed lakes or lagoons without any contact with the ocean. The emerging (eventually homochiral) peptides should progressively have begun to act as catalysts (Pascal 2003), which may have helped the emergence of self-replicating systems. In addition, the role of NO in the metabolism of currently living organisms, being the remnant of a past, much more important contribution (e.g. as a source of either organic nitrogen or energy), raises questions about a possible key role of this compound in prebiotic chemistry.

Although several questions remain unanswered, such as the effective abundance of NO$\textsubscript{x}$ in the atmosphere, or of cyanate in the hydrosphere in regard to the requirements of the primary pump, this model presents the unique advantage to allow experimental testing. This scenario should work as well with NCA produced by other prebiotic pathways. To our opinion, NCA should be considered as a major prebiotic intermediate.

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